Cloned DNA Polymerases from Thermotoga maritima and Mutants Thereof

Abstract

The invention relates to a substantially pure thermostable DNA 5 polymerase from Thermotoga (Tne and Tma) and mutants thereof. The Tne DNA polymerase has a molecular weight of about 100 kilodaltons and is more thermostable than Taq DNA polymerase. The mutant DNA polymerase has at least one mutation selected from the group consisting of (1) a first mutation that substantially reduces or eliminates $3' \rightarrow 5'$ exonuclease activity of said DNA 10 polymerase; (2) a second mutation that substantially reduces or eliminates $5' \rightarrow 3'$ exonuclease activity of said DNA polymerase; (3) a third mutation in the O helix of said DNA polymerase resulting in said DNA polymerase becoming nondiscriminating against dideoxynucleotides. The present invention also relates to the cloning and expression of the wild type or mutant DNA polymerases in 15 E. coli, to DNA molecules containing the cloned gene, and to host cells which express said genes. The DNA polymerases of the invention may be used in wellknown DNA sequencing and amplification reactions.

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